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ILE 'USPAT' ENTERED AT 13:55:27 ON 28 SEP 96
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
  *              W E L C O M E   T O   T H E                      *
  *         U. S.   P A T E N T   T E X T   F I L E               *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
=> s selectin?(p)(combination? or combin?)

        169581 SELECTIN?
        806284 COMBINATION?
       1045639 COMBIN?

L1       18392 SELECTIN?(P)(COMBINATION? OR COMBIN?)
=> s l1 (p(w)selectin? and l(w)selectin?)
MISSING OPERATOR 'L1 (P'
=> s l1 (p)(w)selectin? and l(w)selectin?)
MISSING TERM 'P)(W'
=> s l1(p)(p(w)selectin? and l(w)selectin?)
PROXIMITY OPERATION NOT ALLOWED
=> s (l1)(p)((p(w)selectin? and l(w)selectin?))
PROXIMITY OPERATION NOT ALLOWED
YOU HAVE RECEIVED THIS ERROR MESSAGE  2 CONSECUTIVE TIMES
Certain operators may not be nested in combination with other
operators.  A nested operator is valid only when it occurs at the same
level or above the operator outside the nested phrase as determined by
the following precedence list:
                    1.    Numeric
                    2.    (W), (NOTW), (A), (NOTA)
                    3.    (S), (NOTS)
                    4.    (P), (NOTP)
                    5.    (L), (NOTL)
                    6.    AND, NOT
                    7.    OR
For example, '(MONOCLONAL(W)ANTIBOD?)(L)ANTIGEN?' is valid since (W)
is above (L) on the precedence list.  However,
'((THIN(W)LAYER)(L)PHOSPHOLIPID#)(A)LACTONE#' is not valid since (L)
is below (A) on the precedence list.  The only exception is the 'OR'
operator.  This operator may be used in combination with any other
operator.  For example, '(ATOMIC OR NUCLEAR)(W)REACTOR' is valid.
IF YOU REQUIRE FURTHER HELP, PLEASE CONTACT YOUR LOCAL HELP DESK
=> s l1 and (p(w)selectin?) and (l(w)selectin?)
        532854 P
        169581 SELECTIN?

            65 P(W)SELECTIN?
        466576 L
        169581 SELECTIN?

            92 L(W)SELECTIN?
L2          10 L1 AND (P(W)SELECTIN?) AND (L(W)SELECTIN?)
=> d l2 1-10

1.  5,527,890, Jun. 18, 1996, Derivatives of triterpenoid acids and uses
thereof; Narasinga Rao, et al., 536/5; 424/533; 536/4.1, 4.4, 17.2, 17.4,
17.9, 18.7 [IMAGE AVAILABLE]

2.  5,527,785, Jun. 18, 1996, Selectin receptor modulating compositions;
Michael P. Bevilacqua, et al., 514/56, 54, 61; 536/21 [IMAGE AVAILABLE]
```

3. 5,508,387, Apr. 16, 1996, Selectin binding glycopeptides; Peng C. Tang, et al., 530/403, 322; 536/18.7, 53, 54, 115 [IMAGE AVAILABLE]

4. 5,489,578, Feb. 6, 1996, Sulfated ligands for **l**-**selectin** and methods of treating inflammation; Steven D. Rosen, et al., 514/61, 25, 53, 54, 62; 536/4.1, 17.2, 18.7, 53, 54, 55, 55.1, 55.2 [IMAGE AVAILABLE]

5. 5,484,891, Jan. 16, 1996, Selectin ligands; Laurence A. Lasky, et al., 530/387.3; 435/7.2; 530/350, 395 [IMAGE AVAILABLE]

6. 5,464,935, Nov. 7, 1995, Peptide inhibitors of selectin binding; George A. Heavner, et al., 530/329, 330 [IMAGE AVAILABLE]

7. 5,460,945, Oct. 24, 1995, Device and method for analysis of blood components and identifying inhibitors and promoters of the inflammatory response; Timothy A. Springer, et al., 435/7.24; 422/58, 69; 427/2.11, 2.13; 435/2, 7.23, 7.8, 29, 30, 174, 176, 177, 240.2, 287.1, 287.2, 287.9, 288.3, 288.5 [IMAGE AVAILABLE]

8. 5,444,050, Aug. 22, 1995, Binding of E-selectin or **P**-**selectin** to sialyl Lewis.sup.x or sialyl-Lewis.sup.a; Timothy P. Kogan, et al., 514/25; 536/17.2, 17.3, 17.4, 17.5, 18.4 [IMAGE AVAILABLE]

9. 5,304,640, Apr. 19, 1994, DNA sequence encoding a selectin ligand; Laurence A. Lasky, et al., 536/23.5; 435/69.1, 172.3, 240.2, 320.1 [IMAGE AVAILABLE]

10. 4,718,094, Jan. 5, 1988, Speech recognition system; Lalit R. Bahl, et al., 381/43; 395/2.49, 2.6, 2.61, 2.64, 2.65 [IMAGE AVAILABLE]
=> d l2 1-9 date

L2: 1 of 10

TITLE: Derivatives of triterpenoid acids and uses thereof
US PAT NO: 5,527,890 [IMAGE AVAILABLE] DATE ISSUED: Jun. 18, 1996
APPL-NO: 08/049,018 DATE FILED: Apr. 16, 1993

L2: 2 of 10

TITLE: Selectin receptor modulating compositions
US PAT NO: 5,527,785 [IMAGE AVAILABLE] DATE ISSUED: Jun. 18, 1996
APPL-NO: 08/089,076 DATE FILED: Jul. 7, 1993
REL-US-DATA: Continuation-in-part of Ser. No. 62,957, May 14, 1993, abandoned.

L2: 3 of 10

TITLE: Selectin binding glycopeptides
US PAT NO: 5,508,387 [IMAGE AVAILABLE] DATE ISSUED: Apr. 16, 1996
APPL-NO: 08/102,032 DATE FILED: Aug. 4, 1993

L2: 4 of 10

TITLE: Sulfated ligands for **l**-**selectin** and methods of treating inflammation
US PAT NO: 5,489,578 [IMAGE AVAILABLE] DATE ISSUED: Feb. 6, 1996
APPL-NO: 08/432,849 DATE FILED: May 2, 1996
REL-US-DATA: Continuation of Ser. No. 155,947, Nov. 19, 1993,

abandoned.

L2: 5 of 10

TITLE: Selectin ligands
US PAT NO: 5,484,891 DATE ISSUED: Jan. 16, 1996
[IMAGE AVAILABLE]
APPL-NO: 08/018,994 DATE FILED: Feb. 18, 1993
REL-US-DATA: Division of Ser. No. 834,902, Feb. 13, 1992, Pat. No. 5,304,640, which is a continuation-in-part of Ser. No. 695,805, May 6, 1991, Pat. No. 5,318,890.

L2: 6 of 10

TITLE: Peptide inhibitors of selectin binding
US PAT NO: 5,464,935 DATE ISSUED: Nov. 7, 1995
[IMAGE AVAILABLE]
APPL-NO: 08/384,680 DATE FILED: Feb. 6, 1995
REL-US-DATA: Continuation of Ser. No. 891,986, May 28, 1992, abandoned.

L2: 7 of 10

TITLE: Device and method for analysis of blood components and identifying inhibitors and promoters of the inflammatory response
US PAT NO: 5,460,945 DATE ISSUED: Oct. 24, 1995
[IMAGE AVAILABLE]
APPL-NO: 07/887,444 DATE FILED: May 20, 1992
REL-US-DATA: Continuation-in-part of Ser. No. 707,841, May 30, 1991, abandoned.

L2: 8 of 10

TITLE: Binding of E-selectin or **P**-**selectin** to sialyl Lewis.sup.x or sialyl-Lewis.sup.a
US PAT NO: 5,444,050 DATE ISSUED: Aug. 22, 1995
[IMAGE AVAILABLE]
APPL-NO: 08/235,293 DATE FILED: Apr. 29, 1994

L2: 9 of 10

TITLE: DNA sequence encoding a selectin ligand
US PAT NO: 5,304,640 DATE ISSUED: Apr. 19, 1994
[IMAGE AVAILABLE]
APPL-NO: 07/834,902 DATE FILED: Feb. 13, 1992
REL-US-DATA: Continuation-in-part of Ser. No. 695,805, May 6, 1991.

=> d l2 5,6 kwic

US PAT NO: 5,484,891 [IMAGE AVAILABLE] L2: 5 of 10

SUMMARY:

BSUM(13)

The three members of the LEC-CAM or selectin family of cell adhesion molecules are: **L**-**selectin** (a.k.a. peripheral lymph node homing receptor (pnHR), LEC-CAM-1, LAM-1, gp90.sup.MEL, gp100.sup.MEL, gp110.sup.MEL, MEL-14 antigen, Leu-8 antigen, TQ-1 antigen, DREG antigen), E-selectin (LEC-CAM-2, LECAM-2, ELAM-1) and **P**-**selectin** (LEC-CAM-3, LECAM-3, GMP-140, PADGEM). These receptors will further on be referred to as "selectins". The structures of the selectin family. . .

SUMMARY:

BSUM(14)

The . . . J. Cell Biol. 99, 1535 (1984); Stoolman et al., Blood 70, 1842 (1987)] suggested that the endothelial ligand recognized by **L**-**selectin** is carbohydrate-based. In one series of experiments, Rosen and colleagues demonstrated that the homing receptor-dependent binding of lymphocytes to pln. . .

SUMMARY:

BSUM(15)

The nature of the endothelial molecule(s) recognized by **L**-**selectin** was subsequently probed with a unique recombinant chimera, consisting of the extracellular domain of **L**-**selectin** joined to the hinge, CH2 and CH3 regions of the human IgG1 heavy chain [see WO 91/08298 published 13 Jun.. . . ligand was abolished by treatment of lymph node sections with sialidase, suggesting that a component of the carbohydrate recognized by **L**-**selectin** was sialic acid-like and further accentuated the importance of the lectin domain in **L**-**selectin**-mediated adhesion [Rosen et al., Science (Wash. D.C.) 228, 1005-1007 (1985); Rosen et al. (1989), Supra, and True et al., J. Cell Biol. 11, 2757- 2764 (1990)]. These results demonstrated the specificity of the **L**-**selectin**-immunoglobulin chimera for the pln HEV ligand and established that the ligand expresses carbohydrate residues that are essential for homing receptor-mediated. . .

SUMMARY:

BSUM(17)

Progress has also been made in the identification of ligands for **P**-**selectin**. Larsen et al. [Cell 63, 467 (1990)] have implicated the Lex determinant [Galb1-4(a1-3Fuc)GlcNAc] as an important element of the **P**-**selectin** ligand on myeloid cells. However, sialic acid is also required for full ligand activity, probably in an a2,3 linkage [Corral. . . 172, 1349 (1990); Moore et al., J. Cell Biol. 112, 491 (1991)]. There is a possibility that the ligand for **P**-**selectin** is the same or very similar to that for E-selectin, especially since both selectins bind to a very similar spectrum. . .

SUMMARY:

BSUM(20)

Another object of the invention is to provide purified selectin, specifically **L**-**selectin** ligands.

SUMMARY:

BSUM(27)

Our . . . were distinctive in that they incorporated high amounts of inorganic sulfate into macromolecules. We have, therefore, analyzed the ability of **L**-**selectin**-IgG chimera to precipitate inorganic sulfate-labeled material from lymph nodes labeled with .sup.35 S-sulfate in organ culture. A prominent 50 kD. . . precipitated from lymph nodes

but were not present in any other organ tested. The precipitation of these components with the **L**-**selectin**-IgG chimera was shown to be calcium-dependent, sensitive to both the MEL-14 mAb and specific carbohydrates. This reaction could be abolished. . . Cell. Biol. 107, 1853 (1988)], precipitated both components. A preliminary biochemical analysis revealed that the .about.50 kD and .about.90 kD **L**-**selectin** ligands were trypsin-sensitive glycoproteins, containing predominantly O-linked chains. [See co-pending patent application Ser. No. 07/695,805 filed 6 May 1991, the. . .

SUMMARY:

BSUM(28)

In order to further characterize the nature of the endothelial ligand recognized by **L**-**selectin**, we have taken the novel approach of affinity purifying the sulfated .about.50 kD HEV glycoprotein with the **L**-**selectin**-IgG chimera. The purified glycoprotein has been subjected to N-terminal amino acid sequencing, and this sequence information has been utilized to clone a cDNA encoding the protein component of this **L**-**selectin** ligand. It has been found that the cDNA encodes a novel, highly O-linked (mucin-like) glycoprotein that appears to function as a scaffold that presents carbohydrates to the lectin domain of **L**-**selectin**. Details of the experimental work along with the findings and their interpretation are provided in the examples.

SUMMARY:

BSUM(37)

In another aspect, the present invention concerns an expression vehicle comprising a nucleotide sequence encoding a selectin ligand, preferably an **L**-**selectin** ligand, operably linked to control sequences recognized by a host cell transformed with the vehicle.

SUMMARY:

BSUM(45)

In . . . in need of such treatment a polypeptide as hereinabove defined in an amount effective in blocking the binding of an **L**-**selectin** receptor on a circulating leukocyte to its endothelial ligand.

DRAWING DESC:

DRWD(3)

FIG. 1 illustrates the structures of the selectin (LEC-CAM) family members as determined by cDNA cloning. Illustrated are the structures for **L**-**selectin**, E-selectin and **P**-**selectin**. The lectin, epidermal growth factor (EGF), and multiple short consensus repeats (SCRs) are shown with hypothetical disulfide bond structures as. . . in the mature protein) as well as a hydrophobic transmembrane spanning anchor (TM) and cytoplasmic tail. Two other forms of **P**-**selectin** are also illustrated, one with a deleted scr-7 domain and another with a deleted membrane spanning anchor.

DRAWING DESC:

DRWD(4)

FIG. . . . structure of the genes encoding members of the selectin family. Illustrated are the genomic structures encoding both human and murine **L**-**selectin**, human E-selectin and human **P**-**selectin**. The dark boxes show exons that encode the various structural motifs, including the start codon for the murine gene (ATG),. . . near a locus encoding a family of proteins that all contain variable numbers of the short SCR exon. The murine **L**-**selectin** is also encoded on murine chromosome 1 in a region syntonic to that found in the human chromosome 1 homologue.

DRAWING DESC:

DRWD(5)

FIG. 3A. Illustrates the purification and N-terminal amino acid sequence of the .about.50 kD **L**-**selectin** ligand. The purification of ligand from conditioned medium was monitored by following added .sup.35 S-labeled ligand. Lane A, Starting conditioned. . .

DRAWING DESC:

DRWD(9)

FIG. . . . and B) shows the nucleotide and the encoded amino acid sequence of the core protein of an endothelial ligand for **L**-**selectin**. (SEQ. ID. No.: 2) The unshaded box illustrates a Kozak translational initiation site surrounding the first methionine codon. The dotted. . . underlined amino acid sequence beginning at residue 20 corresponds with the amino acid sequence determined by N-terminal sequencing of the **L**-**selectin** purified ligand (FIG. 3B) with the exception of a THR at position 34 (a MET in the N-terminal sequence). The. . .

DRAWING DESC:

DRWD(10)

FIGS. 5A and 5B show the immunoprecipitation of the **L**-**selectin** purified .about.50 kD ligand by peptide antibodies. In these figures

DRAWING DESC:

DRWD(15)

FIG. 6. Northern blot analysis of the expression of the mRNA encoding the .about.50 kD **L**-**selectin** ligand. A. Total(a) or poly A+ (b, c) RNA was isolated from normal (a,b) or inflamed (c) peripheral lymph nodes,. . . heart, j) spleen, k) brain, and l) kidney and hybridized on Northern blots with C. the cDNA corresponding to the **L**-**selectin** ligand or D. a chicken beta actin cDNA.

DRAWING DESC:

DRWD(16)

FIG. 7. (Parts A and B) In Situ hybridization analysis of the expression of the mRNA encoding the .about.50 kD **L**-**selectin** ligand. Peripheral lymph node sections were hybridized with either an anti-sense (A) or sense (B) hybridization probe corresponding to the **L**-**selectin** ligand cDNA, washed, exposed to emulsion for 6 weeks and developed. The morphology of the HEV is shown with a. . .

DRAWING DESC:

DRWD(17)

FIG. . . . the structure of the .about.50 kD Selectin ligand. Illustrated is one possible model for the structure of the .about.50 kD **L**-**selectin** ligand on the luminal surface of the peripheral lymph node HEV. The extended brush-like regions correspond to O-linked regions I. . .

DETDESC:

DETD(14)

Immunoglobulins . . . Pat. No. 4,444,878; WO 88/03565; and EP 68,763 and references cited therein. Ligand binding protein-stable plasma protein chimeras, and specifically **L**-**selectin**-immunoglobulin chimeras are, for example, disclosed in WO 91/08298 published 13 Jun. 1991. The immunoglobulin moiety in the chimera of the. . .

DETDESC:

DETD(15)

Selectin, such as **L**-**selectin** binding can, for example, be assayed by determining the binding of radiolabeled (e.g. .sup.35 S-labeled) ligands to immobilized receptor-immunoglobulin chimera,. . . can be used to assay ligand binding. For example, EL-4 cells (ATCC TIB39) are known to express high levels of **L**-**selectin** on their surfaces, and can therefore be used in cell adhesion assays for **L**-**selectin** ligands. Adherent cells can be quantitated by lactate dehydrogenase activity [Bradley et al., J. Cell. Biol. 105, 991 (1987)].

DETDESC:

DETD(35)

The . . . molecules with some differences in their amino acid sequences as compared to the native sequence of a selectin, e.g. an **L**-**selectin** ligand. Ordinarily, the variants will possess at least 70% homology with a native selectin ligand, and preferably, they will be. . .

DETDESC:

DETD(42)

Accordingly, . . . the two highly O-glycosylated, serine- and threonine-rich regions (amino acids 42-63 and amino acids 93-122 in FIG.

4) of the **L**-**selectin** ligand amino acid sequence is expected to have more significant effect on the lymphocyte-high endothelial venule interaction than changes in. . . number of O-linked carbohydrate ligands attached to the serine and threonine residues to be appropriately presented to the leukocyte surface-localized **L**-**selectin** lectin domains, thereby mediating the carbohydrate-dependent adhesive interaction. Alterations within these regions are expected to result in molecules the receptor. . .

DETDESC:

DETD(60)

The . . . prepared from tissue believed to possess mRNA for the selectin ligand and to express it at a detectable level. An **L**-**selectin** ligand gene thus may be obtained from a cDNA library prepared from (mesenteric or peripheral) lymph nodes. Genes encoding the. . .

DETDESC:

DETD(62)

An alternative means to isolate the gene encoding a selectin ligand, e.g. an **L**-**selectin** ligand, is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook et al., Supra or. . .

DETDESC:

DETD(64)

A . . . to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues, preferably mammalian lymph node high endothelial venules (**L**-**selectin** ligand), or myeloid cells (E-selectin and **P**-**selectin** ligands). Among the preferred mammals are humans and members of the following orders: bovine, ovine, equine, murine, and rodentia.

DETDESC:

DETD(65)

The . . . actual nucleotide sequence(s) is/are usually based on conserved or highly homologous nucleotide sequences or regions of a selectin ligand, e.g. **L**-**selectin** ligand.

DETDESC:

DETD(66)

The . . . FIG. 4 (SEQ. ID. No.:2) may be used to isolate DNA encoding other selectin ligands or to isolate DNA encoding **L**-**selectin** ligand from another animal species via hybridization employing the methods discussed above. The preferred animals are mammals, particularly human, bovine,. . .

DETDESC:

DETD(68)

The . . . this invention are preferably constructed by mutating the DNA sequence that encodes the protein core of a wild-type selectin, e.g. **L**-**selectin** ligand. Generally, particular regions or sites of the DNA will be targeted for mutagenesis, and thus the general methodology employed. . .

DETDESC:

DETD(138)

A particularly advantageous purification scheme, specifically developed for the purification of the **L**-**selectin** ligand, will be described in Example 1. This method takes advantage of a unique selectin receptor-immunoglobulin chimera (referred to as **L**-**selectin**-IgG), produced by recombinant methods, which is able to precipitate the corresponding (sulfate-labeled) ligand.

DETDESC:

DETD(140)

The . . . invention can be used to block the binding of a corresponding selectin receptor to its native ligand. For example, the **L**-**selectin** ligand effectively blocks the binding of an **L**-**selectin** receptor on a circulating leukocyte to its native ligand on an endothelial cell. This property is useful for treating a. .
.

DETDESC:

DETD(141)

The **selectin** ligands of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the ligand is **combined** in admixture with a pharmaceutically acceptable carrier. Suitable carriers and their formulations are described in Remington's Pharmaceutical Sciences, 16th ed.,. . .

DETDESC:

DETD(155)

Typically . . . for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-**combining** site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-**combining** site having specificity for a **selectin** ligand and another antigen-**combining** site having specificity for a different antigen.

DETDESC:

DETD(165)

Identification of Surface Glycoproteins on Endothelial Cells Recognized

by **L**-**selectin**

DETDESC:

DETD(166)

This example shows that recombinant **L**-**selectin** selectively binds .sup.35 SO.sub.4 -labeled macromolecules from lymph nodes. In particular, two sulfated, fucosylated and sialylated glycoproteins have been identified.

DETDESC:

DETD(169)

B. Identification of the components Adsorbed to **L**-**selectin**-IgG Beads

DETDESC:

DETD(170)

Affi-Gel Protein A (10 .mu.l packed beads) was incubated with 30 .mu.g of either **L**-**selectin**-IgG (WO 91/08298 published 13 Jun. 1991), CD4-IgG (prepared according to Capon et al., Nature 337:525 (1989) or human IgG.sub.1 (Calbiochem, La Jolla, Calif.) in 1 ml of PBS rocking overnight at 4.degree. C. The beads (referred to as **L**-**selectin**-IgG beads, CD4-IgG beads and huIgG-beads) were washed 3X in PBS and once with lysis buffer. The CD4-IgG and huIgG beads. . .

DETDESC:

DETD(171)

The . . . was added to the supernatant at a final concentration of 5 mM, and the supernatant was mixed immediately with either **L**-**selectin**-IgG beads, CD4-IgG beads or huIgG-beads (typically 200 .mu.l of precleared lysate per 10 .mu.l packed beads), and incubated for 4. . .

DETDESC:

DETD(172)

The materials bound to the **L**-**selectin**-IgG beads were solubilized by boiling in SDS in the presence of 2-mercaptoethanol, electrophoresed on SDS-polyacrylamide gels (9 or 10%) and. . . was exchanged into 10 mM CAHPS in PBS on a Centricon 30 unit (Amicon, Danvers, Mass.), followed by incubation with **L**-**selectin**-IgG beads CD4-IgG or huIgG beads as described above. For the analysis of crude lysate, 200 .mu.l of the precleared lysate. . .

DETDESC:

DETD(173)

**L**-**selectin**-IgG beads precipitated a diffuse 50 kD component (apparent molecular weight range is 50 kD-58 kD) from [.sup.35

S]-sulfate-labeled mesenteric lymph. . .

DETDESC:

DETD(174)

**L**-**selectin**-IgG beads precipitated the 50 kD component when calcium was present, but not in its absence. The specificity of the interaction was further examined with the use of MEL-14 mAb. Preincubation of **L**-**selectin**-IgG beads with this antibody completely blocked the binding of the 50 kD band to the beads, whereas a class-matched control antibody (anti-CD45) had no effect. Fucoidin completely blocked the precipitation of the 50 kD component by **L**-**selectin**-IgG beads, while control polysaccharides (chondroitin sulfate B, chondroitin sulfate A, keratan sulfate) were completely inactive. Further, the presence of PPME. . . yeast mannan (mnn 2) had no effect at the same concentration. The precipitation of the minor 90 kD band by **L**-**selectin**-IgG beads was also calcium dependent, inhibitable by MEL-14 mAb, and blocked by fucoidin and PPME.

DETDESC:

DETD(175)

Finally, sialidase treatment of the glycoproteins was found to inhibit binding by **L**-**selectin**-IgG. Thus, sialic acid on the glycoproteins is apparently essential for binding. This result is in agreement with previous characterizations of. . .

DETDESC:

DETD(177)

Purification of the 50 kD **L**-**selectin** ligand for cloning and sequence determination

DETDESC:

DETD(178)

The work described in Example 1 demonstrated that the **L**-**selectin**-IgG chimera could be utilized to biochemically characterize the .about.50 kD sulfated endothelial ligand produced by peripheral and mesenteric lymph nodes.. . . lymph nodes (PLN) are placed into organ culture (S. Watson-unpublished observations). Thus, the initial step in the purification of the **L**-**selectin** ligand for sequence determination was to produce large quantities of medium conditioned by murine PLN. A second observation that allowed for a dramatic purification was that .about.50 kD sulfated **L**-**selectin** ligand was soluble after treatment of conditioned medium with chloroform-methanol. This step resulted in a >350 fold purification of the. . . column, which took advantage of the apparently high content of carbohydrate in this ligand. The final purification step utilized an **L**-**selectin**-IgG chimera affinity column to purify the ligand. This final step assured that the material contained within the .about.50 kD region would correspond to a glycoprotein that could bind with relatively high affinity to **L**-**Selectin**.

DETDESC:

DETD(181)

For . . . medium was added to a 3 ml covalently crosslinked LEC-IgG-protein A-agarose (LEC x protein A-agarose) column prepared with 10 mg **L**-**selectin**-IgG per i ml packed protein A-agarose (Zymed) following the procedure outlined on pages 522-523 of Antibodies. A Laboratory Manual (1988) Harlow and Lane, Cold Spring Harbor Laboratory. After rocking for 6 hours to overnight with **L**-**selectin** x protein A-agarose, the column was washed with 10 volumes Dulbecco's phosphate-buffered saline (PBS) and the purified material (50 kD **L**-**selectin** ligand, a.k.a. GlyCAM) was eluted with 10 ml 4 mM EDTA in PBS. This material was concentrated on a Centricon. . .

DETDESC:

DETD(186)

The . . . sequence was determined by gas-phase microsequencing of the material purified as described in Example 2. The protein eluted from the **L**-**Selectin**-IgG affinity column was run on a 10% SDS-gel, electroblotted onto a Problott membrane (Applied Biosystems Inc.), stained with Coomassie R-250. . .

DETDESC:

DETD(189)

cDNA Cloning and Sequence Analysis of the .about.50 kD **L**-**Selectin** Ligand

DETDESC:

DETD(190)

A . . . secretory pathway. This region is followed by a sequence corresponding almost exactly to that determined by N-terminal sequencing of the **L**-**selectin**-IgG bound material. The signal sequence-processed 132 amino acid protein is extremely rich in serine and threonine, with about 29% of. . .

DETDESC:

DETD(191)

Perhaps . . . the molecular weight of the processed protein was found to be .about.14,154 kD. Since the molecular weight of the isolated **L**-**selectin** ligand is .about.50 kD, this result suggests that .about.70 kD of the glycoprotein mass is O-linked carbohydrate [Carraway and Hull,. . .

DETDESC:

DETD(195)

In order to ultimately prove that the isolated cDNA encodes a sequence

corresponding to the protein backbone of an **L**-**selectin** ligand, we produced peptides derived from the amino acid sequence predicted from the nucleotide sequence of the isolated ligand cDNA. . . antipeptide sera are now designated CAM01, CAM02, CAM05), and each sera was tested for its ability to immunoprecipitate sulfate labeled **L**-**selectin** ligand that was purified by binding to the **L**-**selectin**-IgG chimera as described above.

DETDESC:

DETD(196)

To verify that the cloned protein is the same as the .sup.35 S-labeled material purified from conditioned medium with the **L**-**selectin** IgG chimera, immunoprecipitation of **L**-**selectin**-IgG purified .sup.35 S-labeled material was performed. The following procedure was used for two separate experiments. For the preparation of immunoprecipitation. . . to remove unbound immunoglobulin and only the 25 .mu.l beads remains. 60 .mu.l of PBS containing approximately 6,000 cpm of **L**-**selectin**-IgG purified .sup.35 S-labeled material is added. This is incubated on ice for 3 hours, flicking the tube every 15 minutes.. . . run under non-reducing conditions so that the 50 kD band would not be compressed. (We have previously established that the **L**-**selectin**-IgG purified .sup.35 S-labeled material does not change mobility in an SDS-gel under reducing conditions.) Also, for one tube, the CAM02. . . in order to show specificity of the antibody-antigen interaction. Finally, an irrelevant control peptide antibody against the C-terminus peptide of **L**-**selectin** (called ROSY 1B), also prepared by Caltag using similar protocols, was tested. Both gels were subjected to fluorography with Enhance (New England Nuclear) and autoradiography with Kodak Xar film. CAM02 completely immunoprecipitates the **L**-**selectin**-IgG purified .sup.35 S-labeled material, CAM02 preimmune and ROSY 1B have no effect. The free CAM02 peptide blocks the specific immunoprecipitation.. . .

DETDESC:

DETD(198)

Expression of the **L**-**Selectin** Ligand

DETDESC:

DETD(199)

FIG. 6 shows a Northern blot analysis of the mRNA encoding the .about.50 kD **L**-**selectin** ligand. As can be seen in FIG. 6A, the mRNA is encoded in the poly A+ fraction and corresponds to. . .

DETDESC:

DETD(200)

Analysis of the expression of the mRNA encoding the **L**-**selectin** ligand in a number of different lymphoid and non-lymphoid tissues reveals that this sequence is expressed in a highly tissue-specific. . .

DETDESC:

DETD(201)

In . . . corresponding to the ligand cDNA is synthesized by HEV cells, consistent with previous immunohistochemical data demonstrating the localization of the **L**-**selectin** ligand to this region of the mesenteric and PLN.

DETDESC:

DETD(202)

The data described here are consistent with the hypothesis that an endothelial ligand for **L**-**selectin** is a unique mucin-type glycoprotein. Mucins, by definition, are serine/threonine rich proteins whose molecular weight is predominantly due to O-linked. . . 266:22733 (1991), Porchet et al., Am. Rev. Resp. Dis. 144:S15 (1991)). The high serine and threonine content found in the **L**-**selectin** ligand described here, coupled with the high degree of glycosylation of the protein (.about.70% by molecular weight), suggests that the. . . PLN ligand. The fact that the O-linked carbohydrates appear to be directly involved in the adhesive interactions mediated by the **L**-**Selectin** lectin domain suggests that the role of the protein backbone described here appears to be as a scaffold for carbohydrate. . . novel type of cell adhesion molecule that functions to present carbohydrates in a tissue-specific manner to the lectin domain of **L**-**selectin**. In this way, the regional expression of this "scaffold" may result in regional trafficking of lymphocyte populations.

DETDESC:

DETD(203)

The . . . to present carbohydrates to the lectin domain of a selectin. As shown in the model illustrated in FIG. 6, the **L**-**selectin** ligand may be thought of as a "bottle brush" that extends into the lumen of the HEV. This would allow. . . a large number of O-linked carbohydrate ligands (the bristles on the brush) to be appropriately presented to the lymphocyte surface-localized **L**-**selectin** lectin domain, thus mediating adhesion to the endothelium. The apparent clustering of these carbohydrates into 2 domains on the ligand. . . presented in a polyvalent manner to enhance the binding avidity of the lymphocyte-HEV adhesive interaction. The mucin-like nature of the **L**-**selectin** ligand could thus function to present polyvalent carbohydrate ligands to the **L**-**selectin** lectin domain via an extended, rod-like platform. If accurate, this would define a new mechanism of cell adhesion in the. . .

DETDESC:

DETD(204)

The expression analysis described here suggests that the regulation of regional lymphocyte trafficking mediated by **L**-**selectin** may be due to the tissue specific expression of the ligand mRNA. We found that only those tissues that were previously described as mediating lymphocyte-HEV interactions via **L**-**selectin** expressed high levels of the mRNA for the ligand, although the extremely low level of mRNA in the Peyer's

patch. . . in part, controlled by the transcriptional activation of the ligand mRNA described here, and suggest that exogenous factors may regulate **L**-**selectin**-mediated adhesion by controlling the transcription of the ligand gene. Of course, the protein backbone of the ligand is insufficient to mediate **L**-**selectin** adhesion, and it is possible that the genes controlling the glycosyl-transferases involved in making the carbohydrate ligand(s) found on this. . . in non-HEV cells. Another level of regulation may involve the mechanisms by which the .about.50 kD ligand receives the appropriate **L**-**selectin**-specific carbohydrate side chains while other O-linked glycoproteins do not. The possibility that the **L**-**selectin** ligand described here can be ectopically expressed in chronic or acute inflammatory sites to mediate lymphocyte or neutrophil trafficking remains. . .

DETDESC:

DETD(205)

While it is clear that the .about.50 kD ligand described here readily adheres to **L**-**selectin** via protein-carbohydrate interactions, the mechanism by which this ligand associates with the endothelial cell surface remains to be defined. The. . . suggest that this ligand may be shed in vivo. A number of other cell surface adhesion molecules, including L- and **P**-**selectins**, Johnston et al., Cell 56, 1033 (1989)) and ICAM I (Rothlein et al., J. Immunol. 147:3788 (1991)), have been found. . . [1984]), and it is therefore possible that this domain could function in a similar manner in the case of the **L**-**selectin** ligand. An alternative hypothesis is that the amphipathic helix could interact weakly with another protein that is more tightly associated. . . glycocalyx in a currently ill-defined manner. A final possibility is that there are several HEV ligands that bind to the **L**-**selectin** lectin domain, some of which are tightly associated with the endothelial cell surface, such as the .about.90 kD sulfated ligand. . .

DETDESC:

DETD(206)

The . . . to also express this carbohydrate-like epitope. It is, therefore, possible that other endothelial glycoproteins exist that present carbohydrate to the **L**-**selectin** lectin domain. The development of monoclonal antibody reagents specific for the mucin-like ligand reported here will therefore be of great. . . since they will allow for an assessment of the relative contribution of this glycoprotein versus others as adhesive ligands for **L**-**selectin**-mediated trafficking.

DETDESC:

DETD(207)

The .about.50 kD **L**-**selectin** ligand is the fourth type of molecule that is involved with cell adhesion in the immune system: 1) the leukocyte integrins, 2) their ligands, the immunoglobulin (Ig) superfamily members, 3) the selectins and 4) the .about.50 kD **L**-**selectin** ligand. The integrins, Ig superfamily members, and selectins have all been found to comprise families containing a diversity of related. . .

CLAIMS:

CLMS(1)

What . . .

encoding the protein having the amino acid sequence shown in FIG. 4 (SEQ. ID. No.:2), capable of binding a native **L**-**selectin**.

CLAIMS:

CLMS(4)

4. The polypeptide of claim 1 which is an isolated native **L**-**selectin** ligand.

US PAT NO: 5,464,935 [IMAGE AVAILABLE] L2: 6 of 10

ABSTRACT:
The present invention provides peptides comprising portions of the amino acid sequence at positions 58-61 of **P**-**selectin**. The invention also provides pharmacuetical compositions comprising the peptides of the invention, diagnostic and therapeutic methods utilizing the peptides and. . .

SUMMARY:

BSUM(2)

This invention relates to peptides which inhibit binding of selectins such as **P**-**selectin**, E-selectin and **L**-**selectin**.

SUMMARY:

BSUM(7)

Endothelium . . . characterization and cloning of E-selectin, also known as ELAM-1, is reviewed by Bevilacqua, et al., in Science 243, 1160-1165 (1989). **L**-**selectin**, also known as peripheral lymph node homing receptor, "the murine Mel 14 antigen" "Leu 8" the "Leu 8 antigen" and. . .

SUMMARY:

BSUM(8)

**P**-**selectin**, also known as GMP-140 (granule membrane protein 140) or PADGEM, is a cysteine-rich and heavily glycosylated integral membrane glycoprotein with an apparent molecular weight of 140,000 as assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDSPAGE). **P**-**selectin** was first purified from human platelets by McEver and Martin, J. Biol. Chem. 259:9799-9804 (1984). The protein is present in. . . is rapidly redistributed to the plasma membrane following platelet activation, as reported by Stenberg, et al., (1985). The presence of **P**-**selectin** in endothelial cells and its biosynthesis by these cells was reported by McEver, et al., Blood 70(5) Suppl. 1:355a, Abstract No. 1274 (1987). In endothelial cells, **P**-**selectin** is found in

storage granules known as the Weibel-Palade bodies. (McEver, et al. J. Clin. Invest. 84:92-99 (1989) and Hattori, et al., J. Biol. Chem. 264:7768-7771 (1989)). **P**-**selectin** (also called GMP-140 or PADGEM) has also been reported to mediate the interaction of activated platelets with neutrophils and monocytes. . .

SUMMARY:

BSUM(10)

When . . . fuse with the plasma membrane, the soluble contents of the granules are released to the external environment, and membrane bound **P**-**selectin** is presented within seconds on the cell surface. The rapid redistribution of **P**-**selectin** to the surface of platelets and endothelial cells as a result of activation suggested that this glycoprotein could play an. . .

SUMMARY:

BSUM(11)

This important role has been confirmed by the observation that **P**-**selectin** is a receptor for neutrophils (Geng et al., Nature 343:757-760 (1990); Hamburger and McEver, Blood 75:550-554 (1990)), monocytes (Larsen, et. . . J. Cell Biol. 112:491-499 (1991)), and perhaps a subset of lymphocytes (Moore, et al. J. Cell Biol. 112:491-499 (1991)). Thus, **P**-**selectin** can serve as a receptor for leukocytes following its rapid mobilization to the surfaces of platelets and endothelial cells stimulated. . .

SUMMARY:

BSUM(12)

Peptides derived from **P**-**selectin** are described in U.S. Ser. No. 07/554,199 entitled "Functionally Active Selectin-Derived Peptides" filed Jul. 17, 1990 by Rodger P. McEver. . . and inflammatory responses in a patient wherein a therapeutically effective amount of a peptide capable of blocking leukocyte recognition of **P**-**selectin** is administered to the patient. U.S. Ser. No. 07/554,199 filed July 17, 1990 also discloses that peptide sequences within the lectin domain of **P**-**selectin**, having homology with the lectin domains of other proteins, especially E-selectin and **L**-**selectin**, selectively inhibit neutrophil adhesion to purified **P**-**selectin**, and can therefore be used in diagnostic assays of patients and diseases characterized by altered binding by these molecules, in. . .

SUMMARY:

BSUM(13)

**P**-**selectin**, E-selectin, and **L**-**selectin** constitute the selectin family, based on their related structure and function. E-selectin is not present in unstimulated endothelium. However, when. . . after exposure to cytokines, as reported by Bevilacqua et al., Proc. Natl. Acad. Sci. USA 84:9238-9242 (1987) (in contrast to **P**-**selectin**, which is stored in granules and presented on the cell surface within seconds after activation). E-selectin has been shown to.

. . similar to those of complement-regulatory proteins, a transmembrane domain, and a short cytoplasmic tail. There is extensive sequence homology between **P**-**selectin** and E-selectin throughout both proteins, but the similarity is particularly striking in the lectin and EGF domains.

SUMMARY:

BSUM(14)

Homing . . . the lymphatic tissues where they are exposed to processed antigens. The lymphocytes then reenter the blood through the lymphatic system. **L**-**selectin** contains a lectin domain, an EGF domain, two complement-binding repeats, a transmembrane domain, and a short cytoplasmic tail. **L**-**selectin** also shares extensive sequence homology with **P**-**selectin**, particularly in the lectin and EGF domains.

SUMMARY:

BSUM(15)

Based on a comparison of the lectin domains between P- E- and **L**-**selectin**, it may be possible to select those peptides inhibiting binding of neutrophils to **P**-**selectin** which will inhibit binding of E-selectin, **L**-**selectin** and other homologous molecules, to components of the inflammatory process, or, conversely, which will inhibit only one selectin- mediated binding.

SUMMARY:

BSUM(20)

It is therefore an object of the present invention to provide peptides interacting with cells recognized by selectins, including **P**-**selectin**, E-selectin, and **L**-**selectin**.

SUMMARY:

BSUM(23)

It . . . another object of the present invention to provide peptides for use in diagnostic assays relating to GMP-140, P-, E- or **L**-**selectin**.

SUMMARY:

BSUM(37)

Peptides of Formula I have as their core region portions of the 58-61 amino acid sequence of **P**-**selectin**, with residue 1 defined as the N-terminus of the mature protein after the cleavage of the signal peptide.

SUMMARY:

BSUM(38)

Tests indicate that the peptides of Formula I inhibit the binding of neutrophils to **P**-**selectin** in concentrations of peptide ranging from about 50 to about 1500 .mu.M. Tests also indicate that alterations within the core. . .

DRAWING DESC:

DRWD(2)

FIG. 1 shows the peptides of Formula I to inhibit the adhesion of human neutrophils to purified human **P**-**selectins**.

DETDESC:

DETD(34)

The . . . will be dependent, in part, on whether one or more peptides are administered. A synergistic effect may be seen with **combinations** of peptides from different, or overlapping, regions of the lectin domain, or in **combination** with peptides derived form the EGF domain of **P**-**selectin**.

DETDESC:

DETD(36)

Peptides that are biologically active are those which inhibit binding of neutrophils, monocytes, subsets of lymphocytes or other cells to **P**-**selectin**, or which inhibit leukocyte adhesion to endothelium that is mediated by E-selectin and/or **L**-**selectins**.

DETDESC:

DETD(37)

Peptides can be screened for their ability to inhibit adhesion to cells, for example, neutrophil adhesion to purified **P**-**selectin** immobilized on plastic wells, using the assay described by Geng, et al., Nature 343, 757-760 (1990) .

DETDESC:

DETD(39)

**P**-**selectin** is isolated from human platelet lysates by immunoaffinity chromatography on antibody S12-Sepharose.TM. and ion-exchange chromatography on a Mono-Q.TM. column (FLPC,. . .

DETDESC:

DETD(41)

The . . . minutes at 4.degree. C. The supernatant (0.5M NaCl wash) is removed and saved; this supernatant contains the soluble form of **P**-**selectin**. Care is taken not to remove the top part of the pellet with the supernatant. The pellets are then homogenized. . . minutes at 4.degree. C., the supernatant is removed. The extraction procedure is repeated with the pellet and the supernatant is **combined**

with the first supernatant. The **combined** extracts, which contain the membrane form of **P**-**selectin**, are adjusted to 0.5M NaCl.

DETDESC:

DETD(42)

The . . . the membrane extract (also adjusted to 0.5M NaCl) are absorbed with separate pools of the monoclonal antibody S12 (directed to **P**-**selectin**) previously coupled to Affigel (Biorad) at 5 mg/mL for 2 hours at 4.degree. C. After letting the resins settle, the. . .

DETDESC:

DETD(43)

Bound **P**-**selectin** is eluted from the S12 Affigel with 100 mL of 80% ethylene glycol, 1 mM MES pH 6.0, 0.01% Lubrol. . .

DETDESC:

DETD(44)

**P**-**selectin** is plated at 5 micrograms/mL and the control proteins: human serum albumin (Alb), platelet glycoprotein IIb/IIIa (IIb), yon Willebrand factor. . .

DETDESC:

DETD(46)

Since the selectins have several functions related to leukocyte adherence, inflammation, and coagulation, compounds which interfere with binding of **P**-**selectin**, E-selectin or **L**-**selectin** can be used to modulate these responses.

DETDESC:

DETD(47)

For example, the peptides can be used to competitively inhibit leukocyte adherence by competitively binding to **P**-**selectin** receptors on the surface of leukocytes. This kind of therapy would be particularly useful in acute situations where effective, but. . .

DETDESC:

DETD(54)

Tumor . . . has been well described, suggesting a role for platelets in the spread of some cancers. Recently, it was reported that **P**-**selectin** binds to tumor cells in a variety of human carcinoma tissue sections (colon, lung, and breast), and that **P**-**selectin** binds to the cell surface of a number of cell lines derived from various carcinomas, but not from melanomas. Aruggo,. . .

DETDESC:

DETD(59)

The . . . susceptibility to infections in which leukocytes would have defective binding to platelets and endothelium because of deficient leukocyte ligands for **P**-**selectin**.

DETDESC:

DETD(60)

The . . . be examined, usually leukocytes, are incubated with the labeled peptides and binding assessed by methods described above with antibodies to **P**-**selectin**, or by other methods known to those skilled in the art. If ligands for **P**-**selectin** are also found in the plasma, they can also be measured with standard ELISA or radioimmunoassay procedures, using labeled **P**-**selectin**-derived peptide instead of antibody as the detecting reagent.
=>

The . . . invention can be used to block the binding of a corresponding selectin receptor to its native ligand. For example, the **L**-**selectin** ligand effectively blocks the binding of an **L**-**selectin** receptor on a circulating leukocyte to its native ligand on an endothelial cell. This property is useful for treating a. . .

DETDESC:

DETD(166)

Identification of Surface Glycoproteins on Endothelial Cells Recognized by **L**-**selectin**

DETDESC:

DETD(167)

This example shows that recombinant **L**-**selectin** selectively binds .sup.35 SO.sub.4 -labeled macromolecules from lymph nodes. In particular, two sulfated, fucosylated and sialylated glycoproteins have been identified.

DETDESC:

DETD(170)

B. Identification of the Components Adsorbed to **L**-**selectin**-IgG Beads

DETDESC:

DETD(171)

Affi-Gel Protein A (10 .mu.l packed beads) was incubated with 30 .mu.g of either **L**-**selectin**-IgG (WO 91/08298 published 13 Jun. 1991), CD4-IgG (prepared according to Capon et al., Nature 337:525 (1989) or human IgG (Calbiochem, La Jolla, Ca.) in 1 ml of PBS rocking overnight at 4.degree. C. The beads (referred to as **L**-**selectin**-IgG beads, CD4-IgG beads and huIgG-beads) were washed 3X in PBS and once with lysis buffer. The CD4-IgG and huIgG beads. . .

DETDESC:

DETD(172)

The . . . was added to the supernatant at a final concentration of 5 mM, and the supernatant was mixed immediately with either **L**-**selectin**-IgG beads, CD4-IgG beads or huIgG-beads (typically 200 .mu.l of precleared lysate per 10 .mu.l packed beads), and incubated for 4. . .

DETDESC:

DETD(173)

The materials bound to the **L**-**selectin**-IgG beads were solubilized by boiling in SDS in the presence of 2-mercaptoethanol, electrophoresed

on SDS-polyacrylamide gels (9 or 10%) and. . . was exchanged into 10 mM CAHPS in PBS on a Centricon 30 unit (Amicon, Danvers, Mass.), followed by incubation with **L**-**selectin**-IgG beads CD4-IgG or huIgG beads as described above. For the analysis of crude lysate, 200 .mu.l of the precleared lysate. . .

DETDESC:

DETD(174)

**L**-**selectin**-IgG beads precipitated a diffuse 50 kD component (apparent molecular weight range is 50 kD-58 kD) from [.sup.35 S]-sulfate-labeled mesenteric lymph. . .

DETDESC:

DETD(175)

**L**-**selectin**-IgG beads precipitated the 50 kD component when calcium was present, but not in its absence. The specificity of the interaction was further examined with the use of MEL-14 mAb. Preincubation of **L**-**selectin**-IgG beads with this antibody completely blocked the binding of the 50 kD band to the beads, whereas a class-matched control antibody (anti-CD45) had no effect. Fucoidin completely blocked the precipitation of the 50 kD component by **L**-**selectin**-IgG beads, while control polysaccharides (chondroitin sulfate B, chondroitin sulfate A, keratan sulfate) were completely inactive. Further, the presence of PPME. . . yeast mannan (mnn 2) had no effect at the same concentration. The precipitation of the minor 90 kD band by **L**-**selectin**-IgG beads was also calcium dependent, inhibitable by MEL-14 mAb, and blocked by fucoidin and PPME.

DETDESC:

DETD(176)

Finally, sialidase treatment of the glycoproteins was found to inhibit binding by **L**-**selectin**-IgG. Thus, sialic acid on the glycoproteins is apparently essential for binding. This result is in agreement with previous characterizations of. . .

DETDESC:

DETD(178)

Purification of the 50 kD **L**-**selectin** Ligand for Cloning and Sequence Determination

DETDESC:

DETD(179)

The work described in Example 1 demonstrated that the **L**-**selectin**-IgG chimera could be utilized to biochemically characterize the .about.50 kD sulfated endothelial ligand produced by peripheral and mesenteric lymph nodes.. . . lymph nodes (PLN) are placed into organ culture (S. Watson-unpublished observations). Thus, the initial step in the purification of the **L**-**selectin** ligand for

sequence determination was to produce large quantities of medium conditioned by murine PLN. A second observation that allowed for a dramatic purification was that .about.50 kD sulfated **L**-**selectin** ligand was soluble after treatment of conditioned medium with chloroform-methanol. This step resulted in a >350 fold purification of the. . . column, which took advantage of the apparently high content of carbohydrate in this ligand. The final purification step utilized an **L**-**selectin**-IgG chimera affinity column to purify the ligand. This final step assured that the material contained within the .about.50 kD region would correspond to a glycoprotein that could bind with relatively high affinity to **L**-**Selectin**.

DETDESC:

DETD(182)

For . . . The precleared medium was added to a 3 ml covalently crosslinked LEC-IgG-protein A-agarose (LEC.times.protein A-agarose) column prepared with 10 mg **L**-**selectin**-IgG per 1 ml packed protein A-agarose (Zymed) following the procedure outlined on pages 522-523 of Antibodies. A Laboratory Manual (1988) Harlow and Lane, Cold Spring Harbor Laboratory. After rocking for 6 hours to overnight with **L**-**selectin** x protein A-agarose, the column was washed with 10 volumes Dulbecco's phosphate-buffered saline (PBS) and the purified material (50 kD **L**-**selectin** ligand, a.k.a. GlyCAM) was eluted with 10 ml 4 mM EDTA in PBS. This material was concentrated on a Centricon. . .

DETDESC:

DETD(187)

The . . . sequence was determined by gas-phase microsequencing of the material purified as described in Example 2. The protein eluted from the **L**-**Selectin**-IgG affinity column was run on a 10% SDS-gel, electroblotted onto a Problott membrane (Applied Biosystems Inc.), stained with Coomassie R-250. . .

DETDESC:

DETD(190)

cDNA Cloning and Sequence Analysis of the .about.50 kD **L**-**Selectin** Ligand

DETDESC:

DETD(191)

A . . . secretory pathway. This region is followed by a sequence corresponding almost exactly to that determined by N-terminal sequencing of the **L**-**selectin**-IgG bound material. The signal sequence-processed 132 amino acid protein is extremely rich in serine and threonine, with about 29% of. . .

DETDESC:

DETD(192)

Perhaps . . . the molecular weight of the processed protein was found to be .about.14,154 kD. Since the molecular weight of the isolated **L**-**selectin** ligand is .about.50 kD, this result suggests that .about.70 kD of the glycoprotein mass is O-linked carbohydrate [Carraway and Hull,. . .

DETDESC:

DETD(196)

In order to ultimately prove that the isolated cDNA encodes a sequence corresponding to the protein backbone of an **L**-**selectin** ligand, we produced peptides derived from the amino acid sequence predicted from the nucleotide sequence of the isolated ligand cDNA. . . antipeptide sera are now designated CAM01, CAM02, CAM05), and each sera was tested for its ability to immunoprecipitate sulfate labeled **L**-**selectin** ligand that was purified by binding to the **L**-**selectin**-IgG chimera as described above.

DETDESC:

DETD(197)

To verify that the cloned protein is the same as the .sup.35 S-labeled material purified from conditioned medium with the **L**-**selectin** IgG chimera, immunoprecipitation of **L**-**selectin**-IgG purified .sup.35 S-labeled material was performed. The following procedure was used for two separate experiments. For the preparation of immunoprecipitation. . . to remove unbound immunoglobulin and only the 25 .mu.l beads remains. 60 .mu.l of PBS containing approximately 6,000 cpm of **L**-**selectin**-IgG purified .sup.35 S-labeled material is added. This is incubated on ice for 3 hours, flicking the tube every 15 minutes.. . . run under non-reducing conditions so that the 50 kD band would not be compressed. (We have previously established that the **L**-**selectin**-IgG purified .sup.35 S-labeled material does not change mobility in an SDS-gel under reducing conditions.) Also, for one tube, the CAM02. . . in order to show specificity of the antibody-antigen interaction. Finally, an irrelevant control peptide antibody against the C-terminus peptide of **L**-**selectin** (called ROSY IB), also prepared by Caltag using similar protocols, was tested. Both gels were subjected to fluorography with Enhance (New England Nuclear) and autoradiography with Kodak Xar film. CAM02 completely immunoprecipitates the **L**-**selectin**-IgG purified .sup.35 S-labeled material, CAM02 preimmune and ROSY IB have no effect. The free CAM02 peptide blocks the specific immunoprecipitation.. . .

DETDESC:

DETD(199)

Expression of the **L**-**Selectin** Ligand

DETDESC:

DETD(200)

FIG. 6 shows a Northern blot analysis of the mRNA encoding the .about.50 kD **L**-**selectin** ligand. As can be seen in FIG. 6A, the mRNA is encoded in the poly A+ fraction and corresponds to. . .

DETDESC:

DETD(201)

Analysis of the expression of the mRNA encoding the **L**-**selectin** ligand in a number of different lymphoid and non-lymphoid tissues reveals that this sequence is expressed in a highly tissue-specific. . .

DETDESC:

DETD(202)

In . . . corresponding to the ligand cDNA is synthesized by HEV cells, consistent with previous immunohistochemical data demonstrating the localization of the **L**-**selectin** ligand to this region of the mesenteric and PLN.

DETDESC:

DETD(203)

The data described here are consistent with the hypothesis that an endothelial ligand for **L**-**selectin** is a unique mucin-type glycoprotein. Mucins, by definition, are serine/threonine rich proteins whose molecular weight is predominantly due to O-linked. . . 266:22733 (1991), Porchet et al., Am. Rev. Resp. Dis. 144:S15 (1991)). The high serine and threonine content found in the **L**-**selectin** ligand described here, coupled with the high degree of glycosylation of the protein (.about.70% by molecular weight), suggests that the. . . PLN ligand. The fact that the O-linked carbohydrates appear to be directly involved in the adhesive interactions mediated by the **L**-**Selectin** lectin domain suggests that the role of the protein backbone described here appears to be as a scaffold for carbohydrate. . . novel type of cell adhesion molecule that functions to present carbohydrates in a tissue-specific manner to the lectin domain of **L**-**selectin**. In this way, the regional expression of this "scaffold" may result in regional trafficking of lymphocyte populations.

DETDESC:

DETD(204)

The . . . to present carbohydrates to the lectin domain of a selectin. As shown in the model illustrated in FIG. 6, the **L**-**selectin** ligand may be thought of as a "bottle brush" that extends into the lumen of the HEV. This would allow. . . a large number of O-linked carbohydrate ligands (the bristles on the brush) to be appropriately presented to the lymphocyte surface-localized **L**-**selectin** lectin domain, thus mediating adhesion to the endothelium. The apparent clustering of these carbohydrates into 2 domains on the ligand. . . presented in a polyvalent manner to enhance the binding avidity of the lymphocyte-HEV adhesive interaction. The mucin-like nature of the **L**-**selectin** ligand could thus function to present polyvalent carbohydrate ligands to the **L**-**selectin** lectin

domain via an extended, rod-like platform. If accurate, this would define a new mechanism of cell adhesion in the. . .

DETDESC:

DETD(205)

The expression analysis described here suggests that the regulation of regional lymphocyte trafficking mediated by **L**-**selectin** may be due to the tissue specific expression of the ligand mRNA. We found that only those tissues that were previously described as mediating lymphocyte-HEV interactions via **L**-**selectin** expressed high levels of the mRNA for the ligand, although the extremely low level of mRNA in the Peyer's patch. . . in part, controlled by the transcriptional activation of the ligand mRNA described here, and suggest that exogenous factors may regulate **L**-**selectin**-mediated adhesion by controlling the transcription of the ligand gene. Of course, the protein backbone of the ligand is insufficient to mediate **L**-**selectin** adhesion, and it is possible that the genes controlling the glycosyl-transferases involved in making the carbohydrate ligand(s) found on this. . . in non-HEV cells. Another level of regulation may involve the mechanisms by which the .about.50 kD ligand receives the appropriate **L**-**selectin**-specific carbohydrate side chains while other 0-linked glycoproteins do not. The possibility that the **L**-**selectin** ligand described here can be ectopically expressed in chronic or acute inflammatory sites to mediate lymphocyte or neutrophil trafficking remains. . .

DETDESC:

DETD(206)

While it is clear that the .about.50 kD ligand described here readily adheres to **L**-**selectin** via protein-carbohydrate interactions, the mechanism by which this ligand associates with the endothelial cell surface remains to be defined. The. . . [1984]), and it is therefore possible that this domain could function in a similar manner in the case of the **L**-**selectin** ligand. An alternative hypothesis is that the amphipathic helix could interact weakly with another protein that is more tightly associated. . . glycocalyx in a currently ill-defined manner. A final possibility is that there are several HEV ligands that bind to the **L**-**selectin** lectin domain, some of which are tightly associated with the endothelial cell surface, such as the .about. 90 kD sulfated. . .

DETDESC:

DETD(207)

The . . . to also express this carbohydrate-like epitope. It is, therefore, possible that other endothelial glycoproteins exist that present carbohydrate to the **L**-**selectin** lectin domain. The development of monoclonal antibody reagents specific for the mucin-like ligand reported here will therefore be of great. . . since they will allow for an assessment of the relative contribution of this glycoprotein versus others as adhesive ligands for **L**-**selectin**-mediated trafficking.

DETDESC:

DETD(208)

The .about.50 kD **L**-**selectin** ligand is the fourth type of molecule that is involved with cell adhesion in the immune system: 1) the leukocyte integrins, 2) their ligands, the immunoglobulin (Ig) superfamily members, 3) the selectins and 4) the .about.50 kD **L**-**selectin** ligand. The integrins, Ig superfamily members, and selectins have all been found to comprise families containing a diversity of related. . .

CLAIMS:

CLMS(7)

7. The nucleic acid molecule of claim 1 comprising a nucleotide sequence encoding a **L**-**selectin** ligand.

CLAIMS:

CLMS(8)

8. The nucleic acid molecule of claim 7 wherein said **L**-**selectin** ligand is murine.
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6/7/16 (Item 2 from file: 154)
DIALOG(R)File 154:MEDLINE(R)


08414896 96028304
Selective modulation of the expression of L-selectin ligands by an immune response.
Hoke D; Mebius RE; Dybdal N; Dowbenko D; Gribling P; Kyle C; Baumhueter S ; Watson SR
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Curr Biol (ENGLAND) Jun 1 1995, 5 (6) p670-8, ISSN 0960-9822
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Document type: JOURNAL ARTICLE
BACKGROUND: The adhesion molecule L-selectin is expressed on the cell surface of lymphocytes and mediates their migration from the bloodstream into lymph nodes. L-selectin is able to recognize four glycoprotein ligands, three of which--Sgp50, Sgp90, and Sgp200--are sulphated, bind specifically to L-selectin and are synthesized by the high endothelial venules of the peripheral and mesenteric lymph nodes. One of these three sulphated L-selectin ligands, Sgp90, has been shown to be identical to the known surface marker CD34 and is expressed on the cell surface of endothelial cells. The cDNA encoding Sgp50 has been cloned, and its product, which has been designated GlyCAM-1, is secreted. The third ligand, Sgp200, is both secreted and cell-associated. We have investigated how the expression of these sulphated glycoproteins is regulated during an immune response. RESULTS: Here we demonstrated that, during a primary immune response, the expression and secretion of both GlyCAM-1 and Sgp200 are reduced, recovering to normal levels 7-10 days after antigen stimulation. In contrast, the expression of cell-associated CD34 and Sgp200 is relatively unaffected. These results may account for the modest decreases in the binding of an L-selectin-IgG fusion protein to high endothelial venules of inflamed peripheral lymph nodes that have been observed after antigen exposure. In vivo experiments show that, following the decrease in the levels of secreted GlyCAM-1 and Sgp200, migration of lymphocytes from the blood stream into lymph nodes remains L-selectin-dependent, but more lymphocytes home to antigen-primed than unprimed peripheral lymph nodes. CONCLUSIONS: We suggest that the secreted forms of the L-selectin ligands GlyCAM-1 and Sgp200 act as modulators of cell adhesion, and that cell-associated CD34 and Sgp200 are the ligands that mediate the initial loose binding of lymphocytes to high endothelial venules.
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